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Heterogeneity of obesity-asthma association disentangled by latent class analysis, the SAPALDIA cohort

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ABSTRACT

Although evidence for the heterogeneity of asthma accumulated, consensus for definitions of asthma phenotypes is still lacking. Obesity may have heterogeneous effects on various asthma phenotypes. We aimed to distinguish asthma phenotypes by latent class analysis and to investigate their associations with different obesity parameters in adults using a population-based Swiss cohort (SAPALDIA).

We applied latent class analysis to 959 self-reported asthmatics using information on disease activity, atopy, and age of onset. Associations with obesity were examined by multinomial logistic regression, after adjustments for age, sex, smoking status, educational level, and study centre. Body mass index, percent body fat, waist hip ratio, waist height ratio, and waist circumference were used as obesity measure.

Four asthma classes were identified, including persistent multiple symptom-presenting asthma (n = 122), symptom-presenting asthma (n = 290), symptom-free atopic asthma (n = 294), and symptom-free non-atopic asthma (n = 253). Obesity was positively associated with symptom-presenting asthma classes but not with symptom-free ones. Percent body fat showed the strongest association with the persistent multiple symptom-presenting asthma.

We observed heterogeneity of associations with obesity across asthma classes, indicating different asthma aetiologies.

1. Introduction

Asthma is a highly heterogeneous disease with common pathophysiological features including airway hyperresponsiveness and airway inflammation but also with divergent features distinctive of asthma subtypes [1]. Non-eosinophilic asthma, characterized by an absence of eosinophils in the airway inflammation, differs from eosinophilic asthma in many aspects [2]. Non-eosinophilic asthma is more likely to be refractory to corticosteroid therapy and to be non-atopic, whereas epithelial hyperplasia or hypertrophy occurs only in the eosinophilic subtype. This indicates that the variable phenotypes presumably have distinct aetiologies. Recent findings from the Genome Wide Association Studies (GWAS) also suggest that early-onset asthma has distinct genetic risk factors in comparison to the late-onset subtype [3]. Distinguishing asthma phenotypes allows for the examination of the aetiology and pathobiology of the disease and may also contribute to a better prediction of disease progression and more targeted therapies.

Previous studies reported association between obesity and incident asthma [4–7]. However, few studies were designed so that obesity preceded true asthma onset. Asthma can often be unnoticed or undiagnosed for a while. This hinders ensuring that obesity precedes the true incidence of asthma. Therefore, despite the accumulated reports on the association, causality remains inconclusive.

While body mass index (BMI) is the most widely used obesity

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measure, it might not be the optimal measure regarding its role in pathophysiology for respiratory diseases such as asthma. BMI cannot distinguish fat mass from muscular mass, and hence cannot capture one of the most important features of obesity – body fat distribution. Moreover, the relationship between obesity and asthma may be heterogeneous across different asthma phenotypes [8–11].

Latent class analysis (LCA) has been successfully applied to distinguish asthma phenotypes [12–15]. LCA is a method to analyse the relationships among manifest variables, assuming some unobserved categorical variables [16]. In this study, we applied LCA to distinguish asthma phenotypes.

We examined the association between a variety of obesity measures – BMI, percent body fat (PBF), waist circumference (WC), waist hip ratio (WHR), waist height ratio (WHtR) – and different asthma classes found by LCA, utilizing the Swiss Cohort Study on Air Pollution and Lung and Heart Diseases in Adults (SAPALDIA).

2. Methods

2.1. Study population

The Swiss Cohort Study on Air Pollution and Lung and Heart Diseases in Adults (SAPALDIA) was initiated in 1991 (SAPALDIA1), recruiting 9651 adults aged 18–62 years [17]. 8047 subjects from the initial cohort participated in the first follow-up in 2001–3 (SAPALDIA2) [18] and 6088 subjects in the second follow-up in 2010–11 (SAPALDIA3). At each survey, participants underwent a spirometry examination and a detailed in-person interview on respiratory health and risk factors. The subjects who participated at baseline and at least in one follow-up were included in this study (Fig. 1). Ethical approval was obtained from the Swiss Academy of Medical Sciences and the regional committees for each study centre.

2.2. Asthma definition

Subjects were considered to be asthmatic if they answered 'yes' to the question 'Have you ever had asthma?' either at baseline or in the first or the second follow-up (n = 1094). After exclusion of asthmatics with missing information for skin prick test, self-reported nasal allergy, or age of asthma onset (n = 135), LCA was applied to 959 asthmatics. As a sensitivity analysis, we used physician-diagnosed asthma, restricting the sample to 677 asthmatics if they answered 'yes' to both questions 'Have you ever had asthma?' and 'Was this confirmed by a doctor?' either at baseline or in the first or the second follow-up. In an additional sensitivity analysis, we restricted LCA to those who reported either asthma attack in the last 12 months or current asthma medication at least once from baseline to the second follow-up (n = 472).

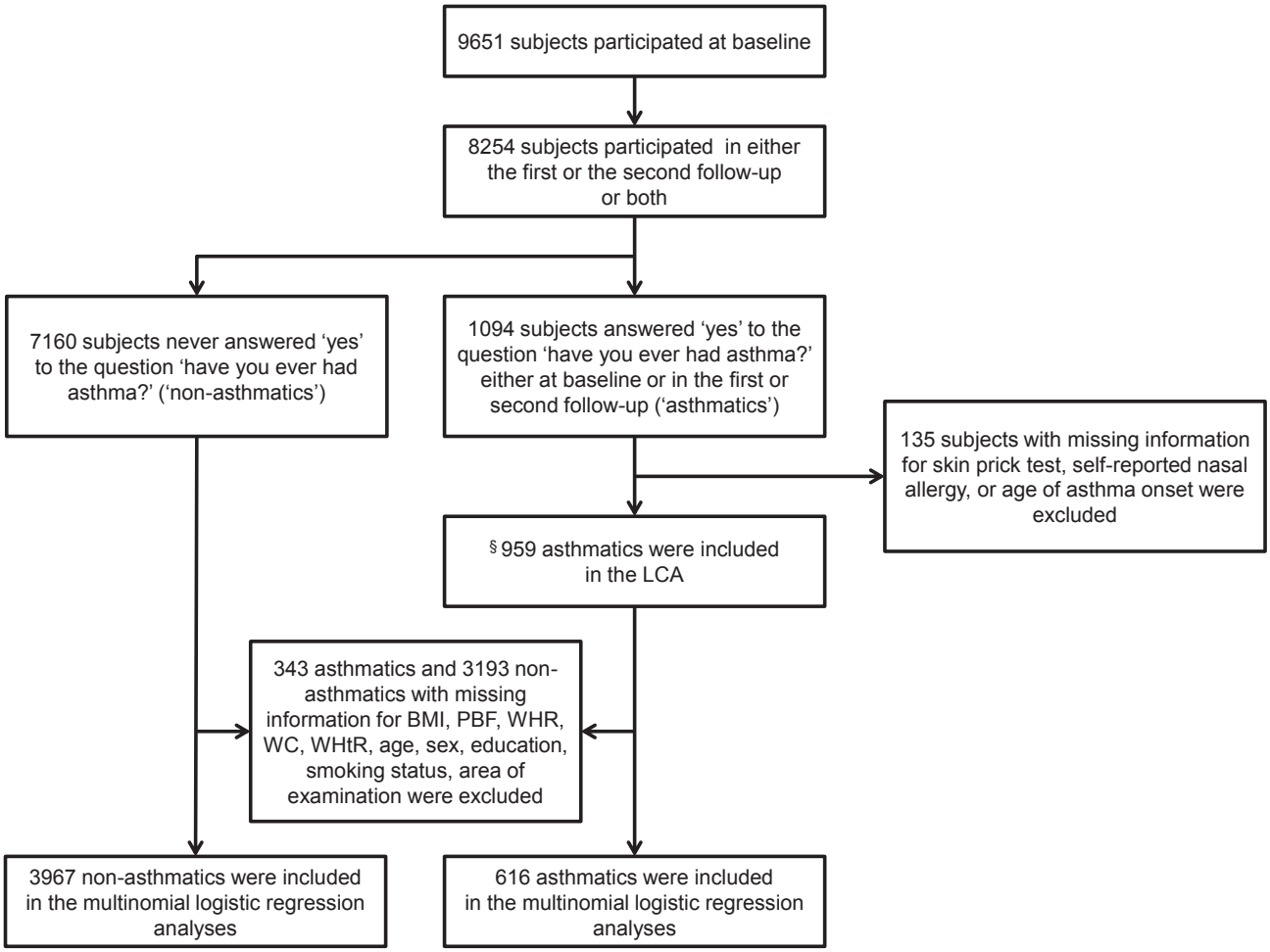

Fig. 1. Flow chart of inclusion and exclusion criteria. § As sensitivity analyses, LCA applied to 677 physician-diagnosed asthmatics instead of 959 self-reported asthmatics or to 472 asthmatics who reported either asthma attack in the last 12 months or current asthma medication at least once from baseline to the second follow-up.

2.3. Obesity measures

We examined five obesity measures including body mass index (BMI; weight in kilograms divided by the square of height in meters), percent body fat (PBF), waist hip ratio (WHR), waist circumference (WC), and waist height ratio (WHtR) in SAPALDIA3. Height was measured in SAPALDIA1, 2, and 3. Weight was asked in SAPALDIA1 and measured in SAPALDIA2 and 3. Waist and hip circumference were measured in SAPALDIA3. Bioelectric impedance was measured in SAPALDIA3 using the device Helios (Helios, Forana, Frankfurt, Germany). Fat-free mass was derived from the measured resistance and reactance using the formula of Kyle et al. [19]. Fat mass was then computed as the difference between body weight and estimated fat-free mass. PBF was defined as the ratio of fat mass to body weight in percent.

2.4. Clustering asthma classes using LCA

Seven variables were chosen as manifest variables to reflect different aspects of asthma phenotypes: 1) asthma attack in the last 12 months (yes or no). SAPALDIA3 information on current asthma attack, current asthma medication, and current asthma symptoms was given priority and then complemented with the information from SAPALDIA2 for those who did not participate in SAPALDIA3; 2) current asthma medication (yes or no); 3) number of asthma symptoms in the last 12 months (no symptoms, one or two symptoms, or more than two symptoms). Five typical respiratory symptoms were considered: breathless while wheezing, chest tightness, shortness of breath at rest, shortness of breath after exercise, and woken by shortness of breath at night. The asthma symptom variables were constructed by counting positive answers across five symptoms and throughout study follow-ups, regardless the number of non-missing answers; 4) number of asthma symptoms repeatedly reported from baseline to the second follow-up (no persistent symptoms, one or two persistent symptoms, or more than two persistent symptoms); 5) atopy defined by positive skin prick test at baseline (yes or no), identified by an adjusted mean wheal diameter $\geq$ 3 mm to at least one of eight common allergens (cat fur, dog epithelia, house dust mite (*Dermatophagoides pteronyssinus*), timothy grass pollen, birch pollen, *Parietaria* pollen, and the moulds *Alternaria* and *Cladosporium*) [18,20]; 6) nasal allergy including hay fever reported at least once from baseline to the second follow-up (yes or no); 7) age of asthma onset $\geq$ 16 or < 16 years (late or early onset), following Moffatt et al. [3]. The cut-off of 16 years is the time around which boys and girls attain puberty and around puberty gender disproportionate incidence rates reverse from male to female preponderance.

LCA was applied to asthmatics with non-missing information on allergy and age of onset (n = 959). For asthma attack in the last 12 months and current asthma medication, subjects with missing information were assumed to be negative. In order to find the appropriate number of latent classes, models were fitted with 2–8 latent classes. The best number was selected primarily based on the Bayesian information criterion (BIC) while the prevalence of classes was also considered. Without compromising too much on BIC, the number of latent classes resulting in more evenly distributed classes was chosen. Each subject was assigned to the latent class with the highest posterior probability.

A descriptive analysis was conducted by examining distributions across LCA-derived asthma classes of age, sex, obesity, education level, smoking status, physical activity, high-sensitive C-reactive protein (hs-CRP) level, airway obstruction, and lung function at baseline including forced expiratory volume in 1 s (FEV_1) as percentage of the predicted, forced vital capacity (FVC) as percentage of the predicted, FEV_1/FVC, forced expiratory flow between 25% and 75% of FVC (FEF_{25-75}) as percentage of the predicted, and bronchial hyperresponsiveness. hs-CRP was measured at SAPALDIA2. Extreme hs-CRP values, i.e. higher than 10 mg/L, were excluded. Airway obstruction was defined as FEF_1/FVC <0.7 according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) [21]. BHR was defined by 20% decline in FEV_1 on methacholine challenge, taking saline as reference. Lung function measurements were obtained using pre-bronchodilator spirometry as previously described [17]. The predicted values for FEV_1, FVC, and FEF_{25-75} were obtained using Brändli et al. equations [22,23].

2.5. Obesity-asthma association examined by multinomial logistic regression

LCA-derived asthma classes and non-asthmatics as reference were regressed on one of the five different obesity measures, adjusting for age, sex, smoking status, education level and study centre. To enable comparison across different obesity measures, odds ratios (OR) were computed for 1 standard deviation (SD) increase. For interpretation purposes, we also reported ORs for overweight or obesity, following commonly used categorization (Table S2). Men were classified as obese if BMI $\geq$ 30 kg/m^2, WHR $\geq$ 1.0, WC $\geq$ 102 cm, or WHtR $\geq$ 0.6 and as overweight if BMI $\geq$ 25 kg/m^2, PBF > 25%, WHR $\geq$ 0.9, WC $\geq$ 94 cm, or WHtR $\geq$ 0.5 but not obese. Women were classified as obese if BMI $\geq$ 30 kg/m^2, WHR $\geq$ 0.85, WC $\geq$ 88 cm, or WHtR $\geq$ 0.6 and as overweight if BMI $\geq$ 25 kg/m^2, PBF > 32%, WHR $\geq$ 0.8, WC $\geq$ 80 cm, or WHtR $\geq$ 0.5 but not obese. Although PBF higher than 25% for men and 32% for women is generally considered overweight, the consensus for optimal cut-offs of PBF is lacking.

2.6. Additional analyses

In an attempt to examine the effect of chronic exposure to obesity, a multinomial logistic regression model was fitted to the stably overweight participants defined as being overweight (BMI $\geq$ 25 kg/m^2) from baseline to the second follow-up. Another sensitivity analysis was conducted, restricting to physically active participants. Subjects were defined as physically active if they reported either moderate physical activity $\geq$ 150 min/week, vigorous physical activity $\geq$ 60 min/week, or combined duration (duration of moderate physical activity + 2 $\times$ duration of vigorous physical activity) $\geq$ 150 min/week. Information on physical activity was obtained from four questions assessing frequency and duration of moderate and vigorous activities [24].

2.7. Statistical software

All analyses were conducted using R 3.1.3 [25]. In particular, R packages poLCA [26] and nnet [27] were used for the LCA and multinomial logistic regression, respectively.

3. Results

3.1. Four asthma classes identified by LCA

Although five classes resulted in slightly better BIC, the model with four classes was chosen due to more evenly distributed class membership (Table S1). The LCA with four classes distinguished persistent multiple symptom-presenting asthma (class 1, n = 122), symptom-presenting asthma (class 2, n = 290), symptom-free atopic asthma (class 3, n = 294), and symptom-free non-atopic asthma (class 4, n = 253). Class 1 was characterized by a high probability of experiencing an asthma attack in the last 12 months, currently being on asthma medication, and having persistent

asthma symptoms (Table 1). Class 1 subjects were more likely to have late-onset asthma. Class 2 was characterized by having one or two persistent or current asthma symptoms. Class 3 and class 4 were characterized by experiencing neither current nor persistent asthma symptoms and were distinguished mainly by atopy and nasal allergy: class 3 subjects were more likely to have atopy and nasal allergy, whereas class 4 subjects were predominantly non-atopic and less likely to have nasal allergy. Contrasts in skin prick test were stronger than contrasts in nasal allergy self-report.

The distribution of age, sex, obesity, education level, smoking status, and physical activity did not differ much between the four classes, except that women are over-represented in class 1 (Table 2 and Table S3). Bronchial hyperresponsiveness (BHR) at baseline was more prevalent in class 1, 2 and 3 than in class 4.

Notably, class 1 and 2 showed higher prevalence of airway obstruction. For class 1 and 2, airway obstruction was already observed at baseline. FEV_1% predicted, FEV_1/FVC ratio, and FEF_{25-75}% predicted were lower in comparison to classes 3 and 4. FVC% predicted did not differ much by asthma classes.

The sensitivity analyses, applying LCA to 768 asthmatics who participated in the second follow-up, or restricting LCA to 677 physician-diagnosed asthmatics, resulted in similar class membership (Table S4; Kappa > 0.9 for both). When restricted to 472 asthmatics who ever reported either asthma attack in the last 12 months or current asthma medication, LCA could not distinguish atopic and non-atopic classes among the symptom-free asthmatics (Table S4; Kappa > 0.3). Instead, the symptom-presenting asthma (class 2) was further differentiated into atopic and non-atopic classes. In any case, the class with highest probability of multiple persistent symptoms similar to the class 1 again showed a stronger association with obesity compared to any other classes (data not shown).

3.2. Heterogeneity of obesity-asthma association

Multinomial logistic regression models were fitted to the four LCA-derived asthma classes with non-asthmatics as reference. Participants with any missing values in the five obesity measures were excluded (Fig. 1). Among the five obesity measures examined as continuous determinants, BMI, PBF, WC and WHtR showed a significant association with class 1 (Table 3). PBF showed the strongest association (OR = 1.63 (95% confidence interval (CI): 1.21–2.20) for 1 SD increase) and further adjustment for BMI did not attenuate this (OR = 1.57 (95% CI: 0.96–2.56)). These results imply that in our sample 1%p higher PBF is associated with a 6.1% increased risk of having the class 1 if BMI remains the same. For class 2, all five obesity measures showed a significant positive association. Interestingly, the associations of PBF, WC and WHtR to class 2 became stronger when adjusted for BMI. None of the five obesity measures showed a significant positive association to

Table 1
Class-conditional probabilities for each of the manifest variables.

		class 1	class 2	class 3	class 4
Asthma attack in the last 12 months		58.6	29.4	5.1	4.1
Current asthma medication		56.2	38.2	5.7	7.3
Number of asthma symptoms in the last 12 months	1–2 symptoms	2.1	84.4	19.2	9.2
	>2 symptoms	96.3	0.0	0.7	0.0
Number of asthma symptoms reported at least twice	1–2 symptoms	36.0	72.1	5.5	10.6
	>2 symptoms	57.2	8.0	1.5	1.2
Positive skin prick test at baseline		44.9	48.2	100.0	7.2
Nasal allergy including hay fever		64.6	61.0	85.5	31.4
Age of asthma onset ≥16 years		75.2	66.8	51.7	57.2

All values are presented in per cent. Class 1: persistent multiple symptom-presenting asthma; Class 2: symptom-presenting asthma; Class 3: symptom-free atopic asthma; Class 4: symptom-free non-atopic asthma.

Table 2
Characteristics of four LCA-derived asthma classes.

		persistent multiple symptom-presenting asthma (class 1)		symptom-presenting asthma (class 2)		symptom-free atopic asthma (class 3)		symptom-free non-atopic asthma (class 4)		non-asthmatics	
		men	women	men	women	men	women	men	women	men	women
N		43 (35.2)	79 (64.8)	132 (45.5)	158 (54.5)	164 (55.8)	130 (44.2)	109 (43.1)	144 (56.9)	3458 (48.3)	3702 (51.7)
Age at baseline [years]		40.8 ± 13.5	38.9 ± 10.9	39.9 ± 11.8	41.5 ± 11.6	37.9 ± 11.7	37.3 ± 11.6	41.6 ± 11.2	41.0 ± 11.1	40.8 ± 11.6	41.6 ± 11.5
Education	Low	4 (9.3)	11 (13.9)	7 (5.3)	13 (8.2)	8 (4.9)	11 (8.5)	5 (4.6)	12 (8.4)	193 (5.6)	451 (12.2)
level	Middle	25 (58.1)	50 (63.3)	74 (56.1)	114 (72.2)	77 (47.0)	81 (62.3)	67 (61.5)	106 (74.1)	2098 (60.7)	2617 (70.8)
	High	14 (32.6)	18 (22.8)	51 (38.6)	31 (19.6)	79 (48.2)	38 (29.2)	37 (33.9)	25 (17.5)	1166 (33.7)	630 (17.0)
Smoking	Never smoker	18 (41.9)	31 (39.7)	42 (31.8)	74 (46.8)	68 (41.5)	66 (50.8)	36 (33.0)	66 (45.8)	1145 (33.2)	1823 (49.6)
status	Former smoker	18 (41.9)	30 (38.5)	68 (51.5)	62 (39.2)	62 (37.8)	48 (36.9)	57 (52.3)	52 (36.1)	1420 (41.2)	1114 (30.3)
	Current smoker	7 (16.3)	17 (21.8)	22 (16.7)	22 (13.9)	34 (20.7)	16 (12.3)	16 (14.7)	26 (18.1)	885 (25.7)	742 (20.2)
Physical	Insufficient	11 (32.4)	21 (28.8)	28 (24.3)	42 (29.8)	25 (19.8)	26 (27.7)	13 (14.4)	34 (28.8)	732 (26.6)	851 (31.2)
activity	Sufficient	23 (67.6)	52 (71.2)	87 (75.7)	99 (70.2)	101 (80.2)	68 (72.3)	77 (85.6)	84 (71.2)	2023 (73.4)	1876 (68.8)
hs-CRP* [mg/L]		2.2 ± 2.2	2.1 ± 2.1	1.4 ± 1.5	2.4 ± 2.3	1.5 ± 1.6	1.5 ± 1.5	1.3 ± 1.4	1.9 ± 2.0	1.5 ± 1.7	1.8 ± 1.9
Airway obstruction (GOLD)		16 (57.1)	30 (52.6)	53 (50.5)	60 (48.4)	44 (38.6)	26 (28.6)	35 (43.2)	41 (38.0)	683 (27.9)	500 (21.3)
Lung function	FEV_1% pred.	88.0 ± 17.6	91.6 ± 18.3	86.1 ± 19.5	92.0 ± 14.0	94.8 ± 14.3	97.5 ± 11.6	97.8 ± 14.3	99.0 ± 15.4	99.9 ± 12.9	100.8 ± 13.4
at baseline	FVC% pred.	96.4 ± 11.1	97.0 ± 14.0	95.5 ± 14.1	97.1 ± 13.4	98.5 ± 11.7	99.9 ± 11.7	101.5 ± 11.7	101.3 ± 14.7	100.6 ± 12.4	100.5 ± 13.2
	FEV_1/FVC	0.73 ± 0.11	0.78 ± 0.11	0.73 ± 0.12	0.77 ± 0.08	0.77 ± 0.09	0.80 ± 0.07	0.77 ± 0.08	0.79 ± 0.07	0.79 ± 0.07	0.82 ± 0.07
	FEF_{25-75}% pred.	76.1 ± 38.5	84.8 ± 32.8	76.4 ± 31.0	81.8 ± 29.5	90.3 ± 29.7	91.5 ± 25.4	93.0 ± 33.5	94.0 ± 28.7	100.4 ± 28.8	103.1 ± 28.7
	BHR	13 (68.4)	23 (51.1)	34 (41.5)	58 (55.2)	51 (37.2)	50 (54.9)	18 (22.0)	35 (32.4)	236 (8.6)	506 (18.5)

Data are presented as mean ± standard deviation or number of subjects (%). Unless otherwise noted, information was retrieved from SAPALDIA3 but complemented from SAPALDIA2 for those who did not participate in SAPALDIA3. * Only available in SAPALDIA2. hs-CRP: high-sensitive C-reactive protein; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; FEF_{25-75}: forced expiratory flow between 25% and 75% of FVC, BHR: bronchial hyperresponsiveness.

Table 3
Odds ratio for 1 SD increase in each of five obesity measures after adjustment for age, sex, smoking status, educational level, and area of examination.

	class 1	class 2	class 3	class 4
BMI	1.32	1.23	1.01	1.04
	[1.09, 1.60]	[1.08, 1.41]	[0.85, 1.19]	[0.88, 1.21]
PBF	1.63	1.47	0.96	0.98
	[1.21, 2.20]	[1.21, 1.78]	[0.78, 1.19]	[0.79, 1.21]
adjusted for BMI	1.57	1.49	0.88	0.86
	[0.96, 2.56]	[1.09, 2.04]	[0.63, 1.23]	[0.62, 1.21]
WHR	1.29	1.46	0.98	0.79
	[0.98, 1.71]	[1.23, 1.75]	[0.78, 1.22]	[0.64, 0.98]
adjusted for BMI	1.13	1.38	0.96	0.73
	[0.82, 1.55]	[1.14, 1.68]	[0.75, 1.23]	[0.58, 0.93]
WC	1.40	1.42	1.01	0.93
	[1.10, 1.77]	[1.22, 1.66]	[0.83, 1.21]	[0.77, 1.13]
adjusted for BMI	1.21	1.79	0.99	0.69
	[0.74, 1.97]	[1.30, 2.46]	[0.69, 1.41]	[0.48, 0.98]
WHtR	1.41	1.38	1.03	0.97
	[1.14, 1.75]	[1.19, 1.59]	[0.86, 1.23]	[0.82, 1.16]
adjusted for BMI	1.41	1.73	1.09	0.78
	[0.87, 2.26]	[1.26, 2.38]	[0.76, 1.57]	[0.55, 1.11]

95% confidence intervals are in square brackets. Note that the odds ratios are obtained from multinomial logistic regression with non-asthmatics as reference category, and hence they are conditional on either being non-asthmatic or respective class. Class 1: persistent multiple symptom-presenting asthma; Class 2: symptom-presenting asthma; Class 3: symptom-free atopic asthma; Class 4: symptom-free non-atopic asthma.

symptom-free asthma (classes 3 and 4). WHR was even negatively associated with class 4. Interaction analyses suggested a gender difference in the positive association of obesity with class 1 and the association to be stronger in men, but the results were inconsistent across different obesity measures (data not shown).

Being obese showed a positive association with classes 1 and 2 irrespective of the parameter used for classification (BMI, WHR, WC or WHtR) (Table S5). Being overweight defined by PBF showed strong positive associations with classes 1 and 2, in comparison with being overweight defined by other obesity measures.

3.3. Stronger association among the stably overweight

When the analysis was restricted to participants who were stably overweight (BMI $\geq$ 25 kg/m^2) from baseline to the second follow-up, the association of PBF with persistent multiple symptom-presenting asthma increased (OR = 2.45 (95% CI 1.15–5.21)) (Fig. 2 and Table S6). This corresponds to saying that among the stably overweight, 1% p higher PBF is associated with a 12.4% increased risk of having class 1. BMI, WC and WHtR also showed a stronger association to class 1 when restricted to the stably overweight, but not as pronounced as for PBF. This restricted analysis did not lead to much increase in ORs for class 2.

When the analyses were restricted to physically active participants, the associations were not altered (Table S7).

4. Discussion

LCA enabled us to identify asthma sub-phenotypes in an agnostic way, with a priori selected relevant characteristics taken into consideration. Simple classification, for example by creating a contingency table, would suffer from low power, given the large number of characteristics to consider. Unlike such simple classification, LCA reveals the co-occurrence and importance in distinguishing classes over multiple characteristics. The LCA-derived asthma classes were distinguished mostly by disease activity and atopic status. Our multinomial logistic regression analyses showed that obesity was associated with symptom-presenting asthma classes but not with symptom-free ones, indicating they may indeed have different aetiologies. Associations were consistently strongest for PBF and the highest odds ratios were observed for the association between PBF and class 1 asthma sub-phenotype.

Class 1 represented relatively severe and presumably poorly controlled asthma. Subjects of this class are also more likely to have late-onset, non-atopic asthma and to be female. This finding is in line with results from earlier studies aiming to identify asthma sub-phenotypes by applying various clustering methods [13,28–30]. In contrast to the previous clustering studies, we did not identify age of disease onset to be a key differentiating factor. However, categorization of age-of-onset by 16 years cut-off may not be the optimal way to assess. A recent SAPALDIA study showed that gender difference in asthma incidence attenuated in late adulthood [31] and menopause has been associated with asthma phenotypes [32]. It would be interesting to investigate asthma that manifests later in adulthood as potentially a separate phenotype or to examine if the association to obesity changes around menopause, but limited number of observations did not allow such additional analysis. Our analyses revealed the strongest association of obesity with class 1, pointing to a distinct asthma entity both from a clinical and an aetiological perspective. Although this study assessed self-reported ever asthma, possibly including the asthmatics whose childhood asthma had grown out, class 1 was also identified when LCA was restricted to those who reported either asthma attack or medication during the time of SAPALDIA follow-up and showed the strongest association with obesity.

Most obesity measures examined in this study showed a positive association with the symptom-presenting asthma classes. Comparing the OR for 1 SD increase, PBF had the strongest association with class 1, suggesting that PBF captures the effects of adiposity on respiratory health better than BMI, confirming the limitation of BMI to be used as health-relevant obesity measure. In recognition of the limitation of BMI, Fenger et al. examined various obesity measures in relation to asthma [8] and lung function [33], although they did not report any specific measure being superior to BMI. Wang et al. showed stronger association of asthma to PBF than to BMI among children [34]. Alternatively, this strong association between PBF and symptom-presenting asthma classes might be in part attributed to reverse causation, i.e. asthmatics tend to lack physical activity and lose muscle mass, which then associates with higher PBF.

One of the most favoured hypotheses explaining the obesity-asthma association is that low-grade chronic inflammation induced by visceral adipose tissue leads to airway inflammation. In fact, we did observe higher serum levels of high-sensitive C-

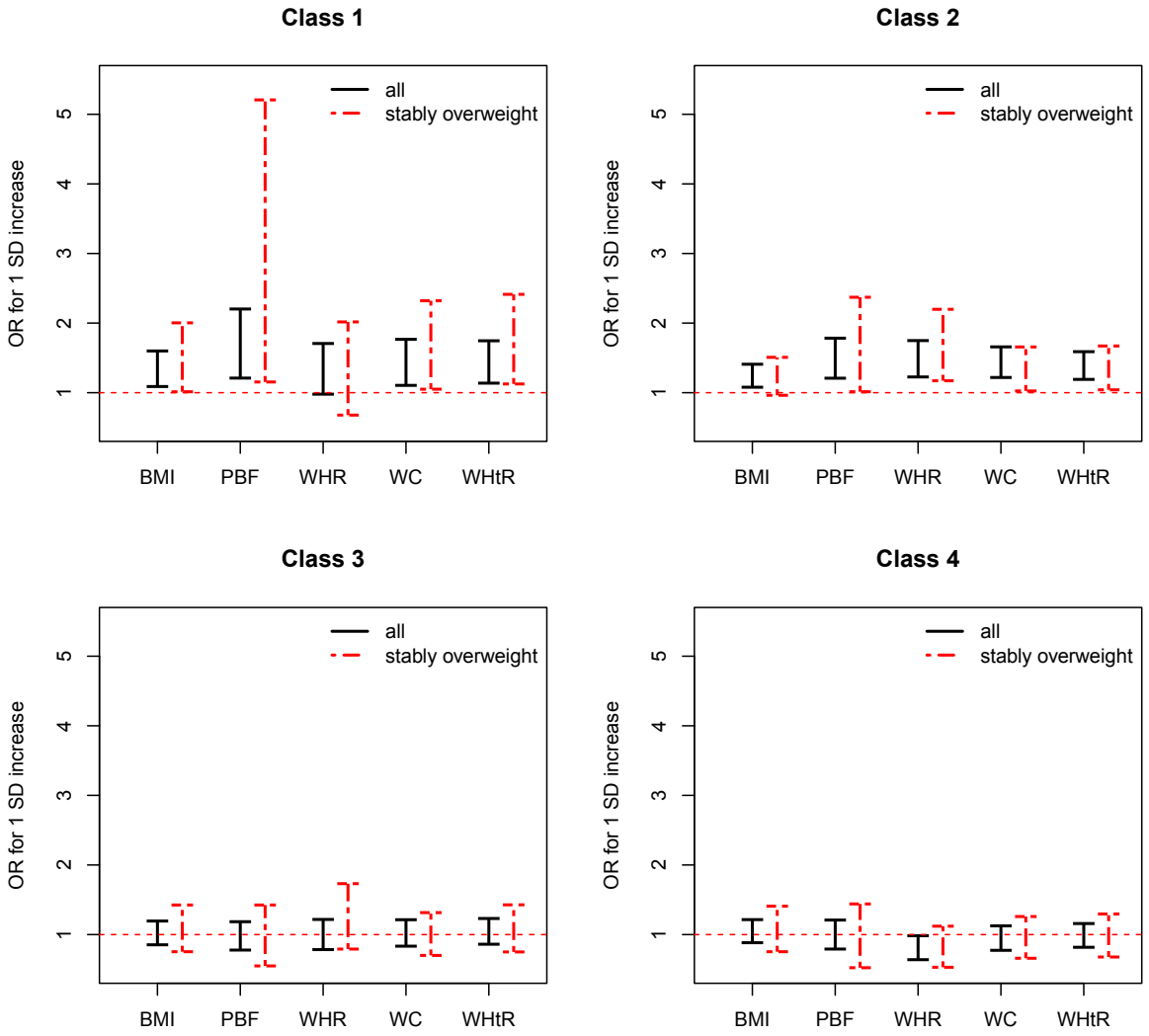

Fig. 2. Odds ratio for 1 SD increase in each of five obesity measures before and after restriction to the stably overweight participants, adjusted for age, sex, smoking status, educational level, and area of examination.

reactive protein (hs-CRP) in symptom-presenting asthma classes (Table 2). While a positive association between hs-CRP and BMI was observed among SAPALDIA participants, ANCOVA with LCA-derived asthma classes as factor and BMI and sex as covariates did not identify asthma classes as a statistically significant determinant of hs-CRP (data not shown). Obese asthmatics have often shown a dissociation between symptoms and biomarkers of airway inflammation such as sputum eosinophil count or exhaled nitric oxide [35,36], suggesting a distinct underlying inflammatory mechanism. A recent study also reported that airway inflammation was not elevated in obese asthmatics [37]. Elucidation of the pathophysiology linking obesity to asthma requires further studies paying attention to the heterogeneity of asthma phenotypes.

Our results might also be biased due to the fact that obese individuals may be over-diagnosed with asthma. Obesity is thought to cause physiological impairments in lung function such as reduced lung volumes and chest wall restriction [38] and dyspnoea caused by obesity-related impairments may be mistaken for asthma [39]. However, in our study, PBF showed a strong association to symptom-presenting asthma phenotypes even if adjusted for BMI. This suggests that the obesity-asthma relationship is not solely attributed to the impaired lung function caused by obesity. Moreover, we also observed decrease in FEF_{25-75}% predicted, but not in FVC% predicted, in symptom-presenting asthma classes, suggesting that obesity-asthma association is likely due to the airway inflammation rather than mechanical impairments. Independent evidence also showed that the risk of asthma over-diagnosis is not higher among obese than non-obese [40].

Nevertheless, reverse causation remains a plausible explanation for the obesity-asthma association. One can suspect that asthmatics gain weight as a side effect of systemic corticosteroids, higher systemic inflammation, or sedentary life style. However, the commonly used asthma treatment, an inhaler, is not generally known to cause systemic side effects [41]. A more obvious hypothesis would be that respiratory symptoms hinder asthmatics from being physically active and hence lead to weight gain. Due to our study design, we cannot demonstrate that obesity preceded true asthma onset. However, the obesity effect observed in this study did not attenuate when the analysis was restricted to physically active participants, suggesting that the observed association cannot entirely be explained by reverse causation. Interaction analyses also showed that physical activity did not modify the effect of obesity on the severe asthma classes, regardless of obesity metrics used (data not shown).

The effects of all five obesity measures became stronger when the analyses were restricted to stably overweight participants. This

seems to support the causality of the association between obesity and persistent multiple symptom-presenting asthma. Recent findings from a Mendelian randomisation approach point to the causality of the association in childhood asthma [42]. However, in order for a conclusive causal inference, further biological and epidemiological studies are required.

5. Conclusion

We demonstrated that LCA is a useful tool to disentangle the heterogeneity of asthma phenotypes. Four LCA-derived asthma classes were distinguished mainly by disease activity and atopic status. We observed heterogeneous associations with obesity across LCA-derived classes, indicating possible aetiological differences. Most obesity measures showed a positive association with symptom-presenting asthma classes but not with symptom-free ones. PBF was better than BMI in explaining persistent multiple symptom-presenting asthma class. The obesity-asthma association was stronger among the stably overweight.

Author contributions

AJ, CS, and NPH developed the research question and designed the study. AJ, CS, and GL conducted the statistical analyses. AJ, MI, SH, EZ, PB, and NPH contributed to the draft of the manuscript. All authors read and approved the final manuscript.

Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.rmed.2017.02.014.

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